Figure 1A

Muc1 Exemplary Protein – (SEQ ID NO:19)

Signal Peptide Cleavage (G|S; A|S)

```
  1    MTPGTQSPFF LLLLLTVLTV VTGSGHASST PGGEKETSAT QRSSVPSSTE KNAVSMTSSV LSSHSPGSGS

 71    STTQGQDVTL APATEPASGS AATWGQDVTS VPVTRPALGS TTPPAHDVTS APDNKPAPGS TAPPAHGVTS

141    APDTRPPPGS TAPPAHGVTS APDTRPPPGS TAPAAHGVTS APDTRPAPGS TAPPAHGVTS APDNRPALAS

211    TAPPVHNVTS ASGSASGSAS TLVHNGTSAR ATTTPASKST PFSIPSHHSD TPTTLASHST KTDASSTHHS

281    TVPPLTSSNH STSPQLSTGV SFFFLSFHIS NLQFNSSLED PSTDYYQELQ RDISEMFLQI YKQGGFLGLS

351    NIKFRPGSVV VQLTLAFREG TINVHDVETQ FNQYKTEAAS RYNLTISDVS VSDVPFPFSA QSGAGVPGWG

421    IALLVLVCVL VALAIVYLIA LAVCQCRRKN YGQLDIFPAR DTYHPMSEYP TYHTHGRYVP PSSTDRSPYE

491    KVSAGNGGSS LSYTNPAVAA TSANL    (SEQ ID NO:19; NM_002456)
```

Post-translational cleavage site (G|S)

Transmembrane region (TMR)

Figure 1B

Muc1 juxtamembrane domain-GST Fusion

GST plus amino acids 337-422 of Muc1

GST-FLQI YKQGGFLGLS NIKFRPGSVV VQLTLAFREG TINVHDVETQ FNQYKTEAAS RYNLTISDVS VSDVPFPFSA
QSGAGVPGWG IA (SEQ ID NO:7)

Synthetic Peptides

Peptide a 21 amino acids (362-382) QLTLAFREGTINVHDVETQFN   (SEQ ID NO:8)
Peptide b 21 amino acids (383-403) QYKTEAASRYNLTISDVSVSD   (SEQ ID NO:9)
Peptide c 21 amino acids (337-357) FLQIYKQGGFLGLSNIKFRPG   (SEQ ID NO:10)
Peptide d 21 amino acids (354-374) FRPGSVVVQLTLAFREGTINV   (SEQ ID NO:11)
Peptide e 19 amino acids (404-422) VPFPFSAQSGAGVPGWGIA   (SEQ ID NO:12)

Figure 2A

Muc16 Exemplary Protein – (SEQ ID NO:20)

```
1      MEHITKIPNE AAHRGTIRPV KGPQTSTSPA SPKGLHTGGT KRMETTTTAL KTTTALKTT  SRATLTTSVY TPTLGTLTPL    AMINO
81     NASRQMASTI LTEMMITTPY VFPDVPETTS SLATSLGAET VLNRESETTA SLVSRSGAER SPVIQTLDVS               TERMINAL
161    SSEPDTTASW VIHPAETIPT VSKTTPNFFH SELDTVSSTA IPTNISPSEL DALTPLVTIS GTDTSTTFPT               DOMAIN
241    LTKSPHETET RTTWLTHPAE TSSTIPRTIP NFSHHESDAT TSSAIPIMTV SPGAEDLVTS QVTSSGTDRN
321    MTIPTLTLSP GEPKTIASLV THPEAQTSSA IPTSTISPAV SRLVTSMVTS ALTNSPGEPA TTVSLVTHPA
401    QTSPTVPWTT SIFFHSKSDT TPSMTTSHGA ESSSAVPTPT VSTEVPGVVT PLVTSSRAVI STTIPILTLS PGEPETTPSM
481    ATSHGEEASS AIPTPTVSPG VPGVVTSLVT SSRAVTSTTI PILTFSLGEP ETTPSMATSH GTEAGSAVPT VLPEVPGMVT
561    SLVASSRAVT STTLPTLTLS PGEPETTPSM ATSHGAEASS TVPTVSPEVP GVVTSLVTSS SGVNSTSIPT LILSPGELET
641    TPSMATSHGA EASSAVPTPT VSPGVSGVVT PLVTSSRAVT STTIPILTLS SSEPETTPSM ATSHGVEASS AVLTVSPEVP
721    GMVTSLVTSS RAVTSTTIPT LTISSDEPET TTSLVTHSEA KMISAIPTLA VSPTVQGLVT SLVTSSGSET SAFSNLTVAS
801    SQPETIDSWV AHPGTEASSV VPTLTVSTGE PFTNISLVTH PAESSSTLPR TTSRFSHSEL DTMPSTVTSP EAESSSAIST
881    TISPGIPGVL TSLVTSSGRD ISATFPTVPE SPHESEATAS WVTHPAVTST TVPRTTPNYS HSEPDTTPSI ATSPGAEATS
961    DFPTITVSPD VPDMVTSQVT SSGTDTSITI PTLTLSSGEP ETTSFITYS  ETHTSSAIPT LPVSPGASKM LTSLVISSGT
1041   DSTTTFPTLT ETPYEPETTA IQLIHPAETN TMVPKTTPKF SHSKSDTTLP VAITSPGPEA SSAVSTTTIS PDMSDLVTSL
1121   VPSSGTDTST TFPTLSETPY EPETTVTWLT HPAETSTTVS GTIPNFSHRG SDTAPSMVTS PGVDTRSGVP TTTIPPSIPG
1201   VVTSQVTSSA TDTSTAIPTL TPSPGEPETT ASSATHPGTQ TGFTVPIRTV PSSEPDTMAS WVTHPPQTST PVSRTTSSFS
1281   HSSPDATPVM ATSPRTEASS AVLTTISPGA PEMVTSQITS SGAATSTTVP TLTHSPGMPE TTALLSTHPR TGTSKTFPAS
1361   TVFPQVSETT ASLTIRPGAE TSTALPTQTI SSLFTLLVTG TSRVDLSPTA SPGVSAKTAP LSTHPGTETS TMIPTSTLSL
1441   GLLETTGLLA TSSSAETSTS TLTLTVSPAV SGLSSASITT DKPQTVTSWN TETSPSVTSV GPPEFSRTVT GTTMTLIPSE
1521   MPTPPKTSHG EGVSPTTILR TTMVEATNLA TTGSSPTVAK TTTFNTLAG  SLFTPLTTPG MSTLASESVT SRTSYNHRSW
1601   ISTTSSYNRR YWTPATSTPV TSTFSPGIST SSIPSSSTA
                                               AT VPFMVPFTLN FTITNLQYEE DMRHPGSRKF NATERELQGL    TANDEM
1681   LKPLFRNSSL EYLYSGCRLA SLRPEKDSSA MAVDAICTHR PDPEDLGLDR ERLYWELSNL TNGIQELGPY TLDRNSLYVN    REPEAT
1761   GFTHRSSMPT TSTPGTSTVD VGTSGTPSSS PSPTAAGPLL MPFTLNFTIT NLQYEEDMRR TGSRKFNTME SVIQGLLKPL    DOMAIN
1841   FKNTSVGPLY SGCRLTLLRP EKDGAATGVD AICTHRLDPK SPGLNREQLY WELSKLTNDI EELGPYTLDR NSLYVNGFTH
1921   QSSVSTTSTP GTSTVDLRTS GTPSSLSSPT/-/
                      IT LLRDIQDKVT TLYKGSQLHD TFRFCLVTNL TMDSVLVTVK ALFSSNLDPS LVEQVFLDKT LNASFHWLGS    CARBOXY
11511  TYQLVDIHVT EMESSVYQPT SSSSTQHFYP NFTITNLPYS QDKAQPGTTN YQRNKRNIED ALNQLFRNSS IKSYFSDCQV    TERMINAL
11591  STFRSVPNRH HTGVDSLCNF SPLARRVDRV AIYEEFLRMT RNGTQLQNFT LDRSSVLVDG YSPNRNEPLT GNSDLPFWAV    DOMAIN
11671  ILIGLAGLLG LITCLICGVL VTTRRRKKEG EYNVQQQCPG YYQSHLDLED LQ
```

Muc16 juxtamembrane domain-GST Fusion

GST plus amino acids 11559-11666 of Muc16

GST-TN YQRNKRNIED ALNQLFRNSS IKSYFSDCQV STFRSVPNRH HTGVDSLCNF SPLARRVDRV AIYEEFLRMT
RNGTQLQNFT LDRSSVLVDG YSPNRNEPLT GNSDLP (SEQ ID NO:13)

Synthetic Peptides

Peptide a	20 amino acids	(11644-11663)	SSVLVDG YSPNRNEPLT GNS	(SEQ ID NO:14)
Peptide b	20 amino acids	(11559-11578)	TN YQRNKRNIED ALNQLFRN	(SEQ ID NO:15)
Peptide c	21 amino acids	(11576-11596)	FRNSS IKSYFSDCQV STFRSV	(SEQ ID NO:16)
Peptide d	23 amino acids	(11595-11617)	SVPNRH HTGVDSLCNF SPLARRV	(SEQ ID NO:17)
Peptide e	28 amino acids	(11618-11645)	DRV AIYEEFLRMT RNGTQLQNFT LDRSS	(SEQ ID NO:18)

Figure 3
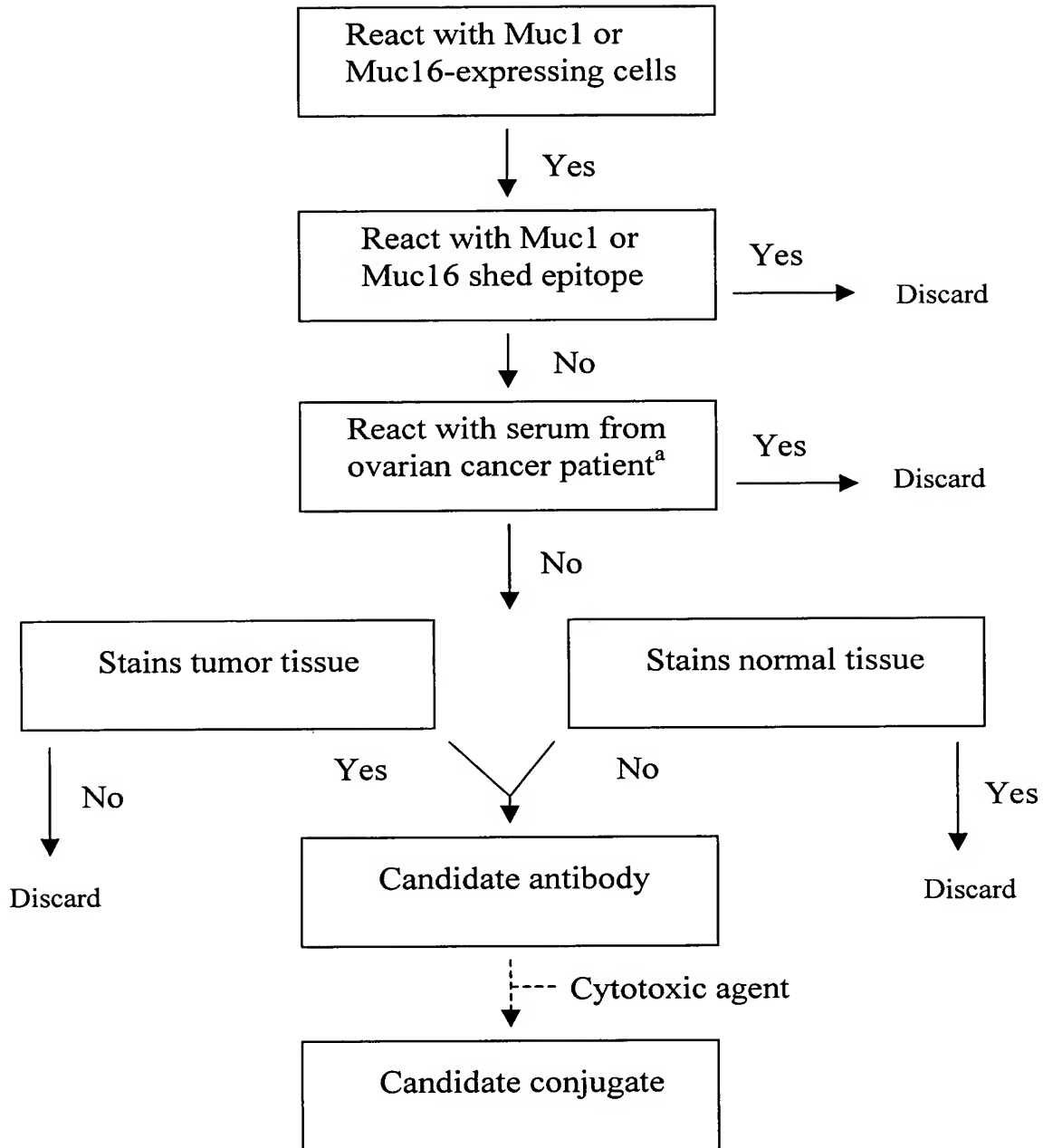
Figure 4A

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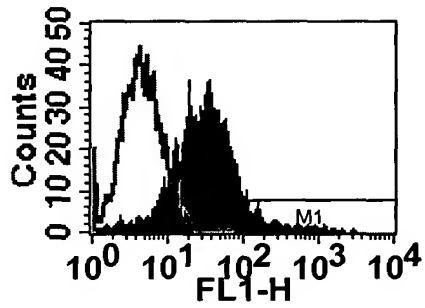
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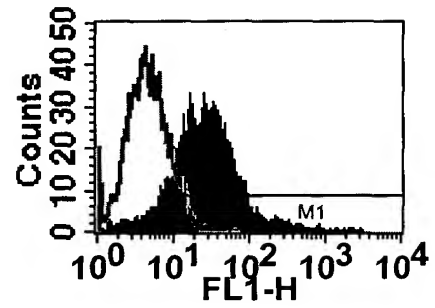
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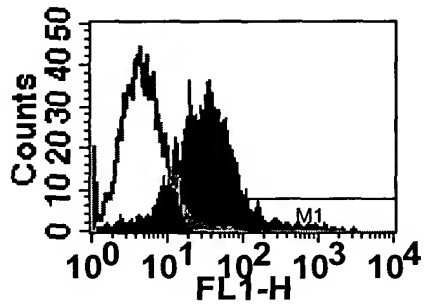
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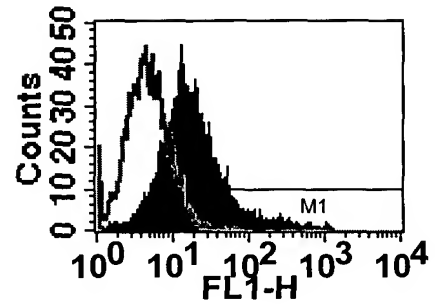
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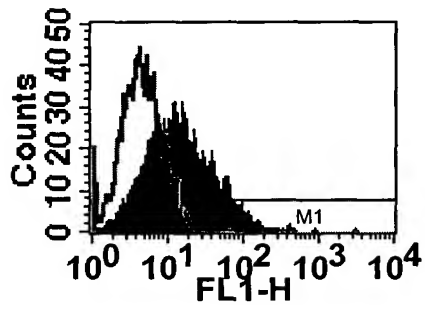
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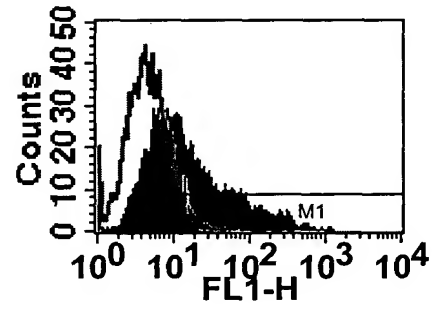
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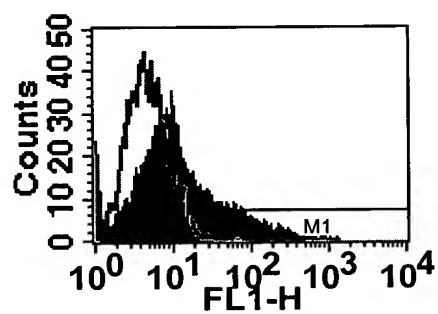
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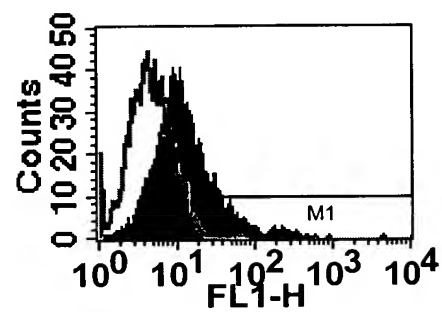
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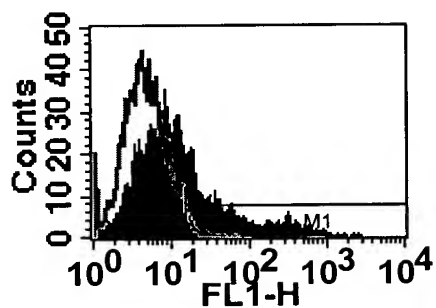
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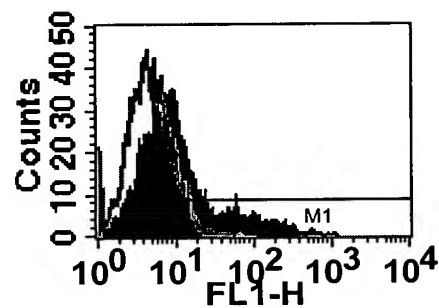
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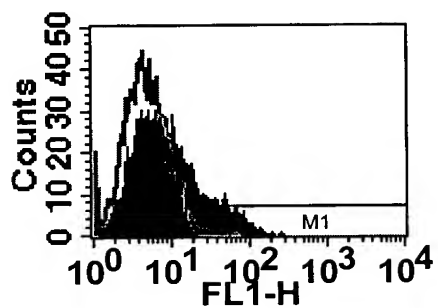
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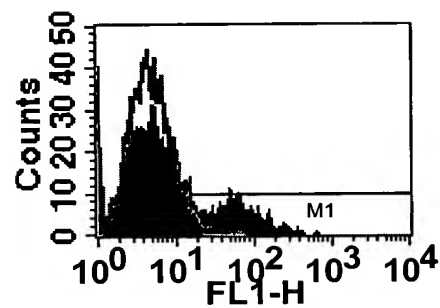
Figure 4B

CM1 (5.36×10^{-8}M)
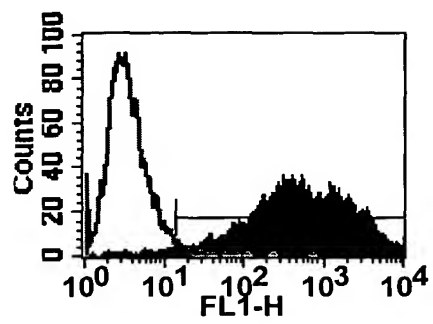
Figure 5A

Clone 1B8
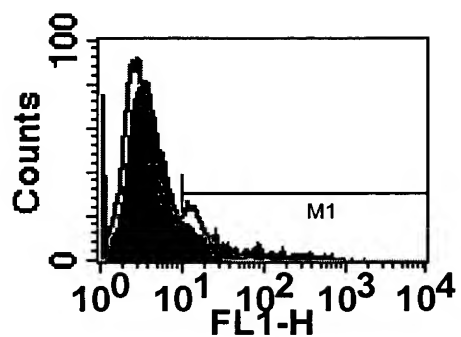
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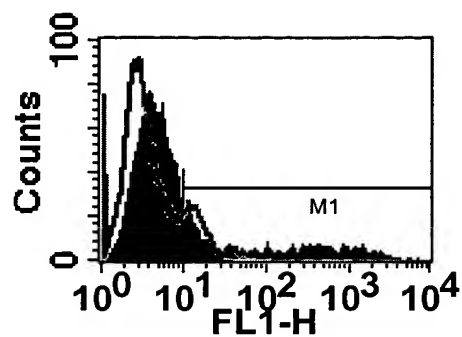
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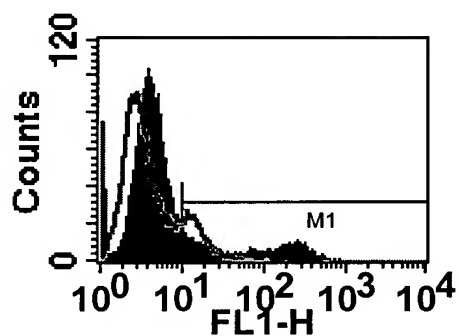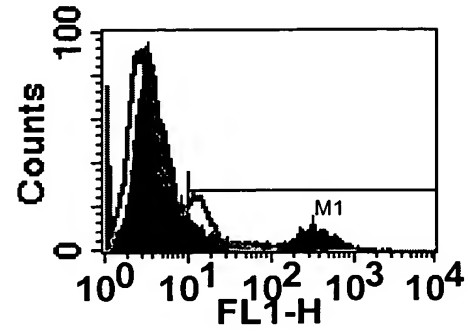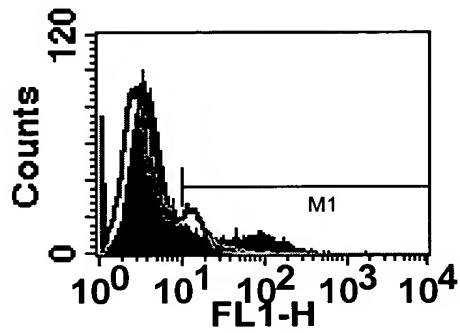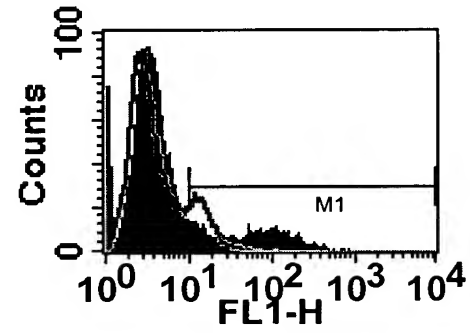
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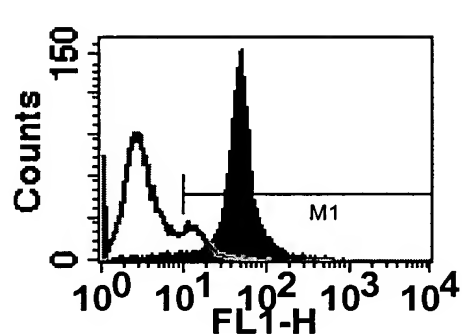
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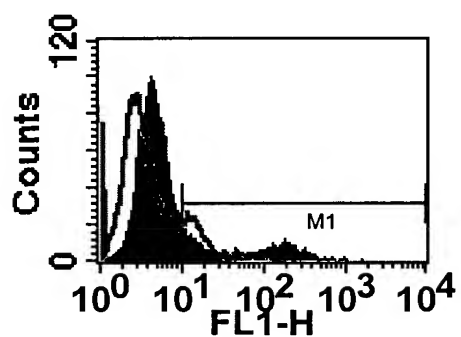
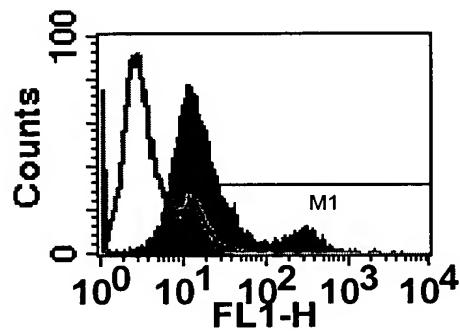Clone 4F8
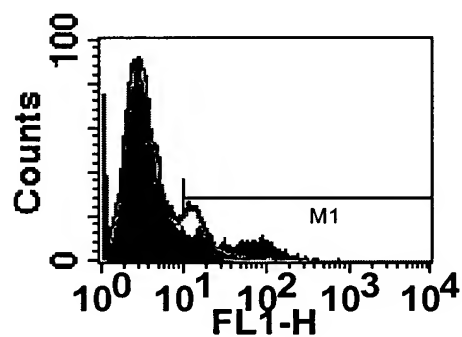
Figure 5A (continued)

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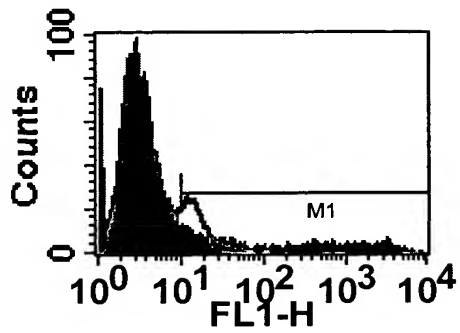
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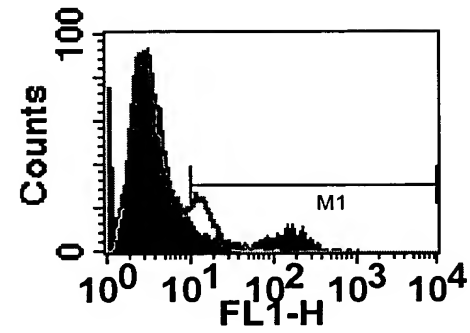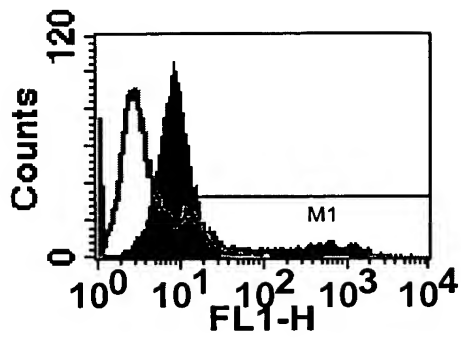

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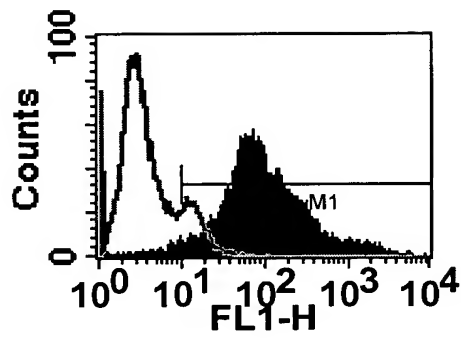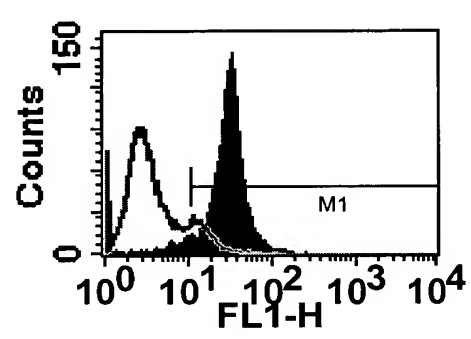

Clone 9G10

Clone 10C3

Clone 10G2

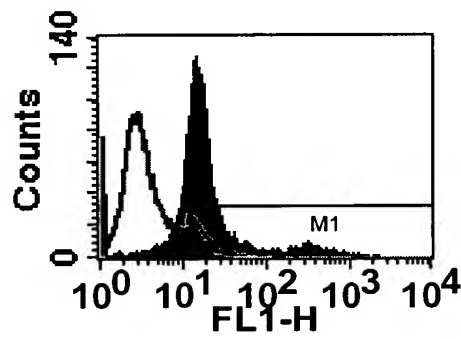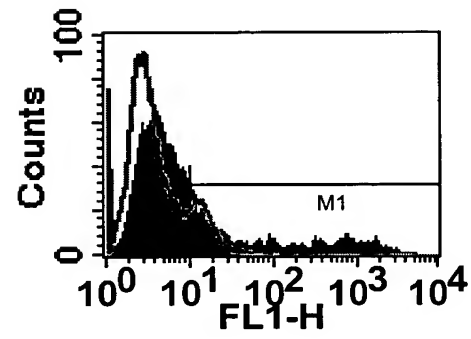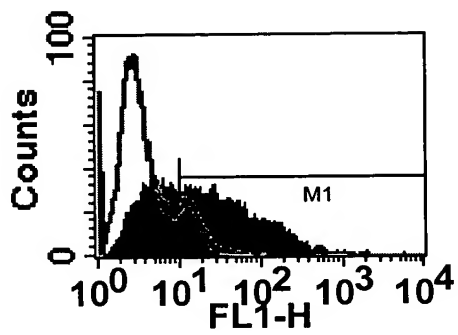# Figure 5A (continued)

Clone 2A9

Clone 2E6
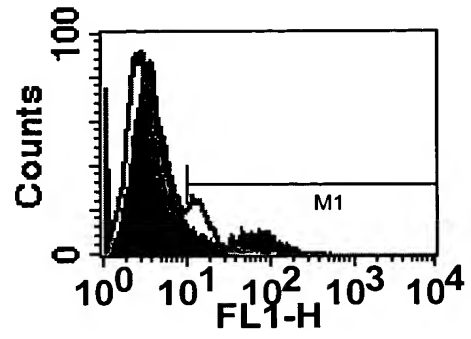
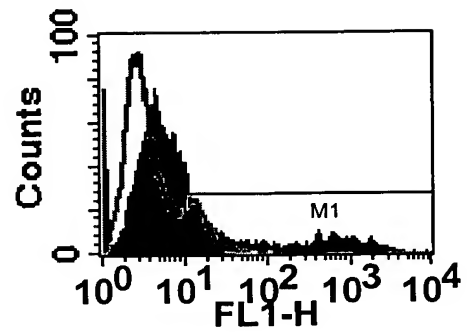Clone 2F9

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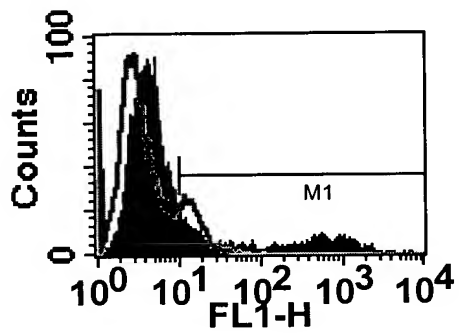
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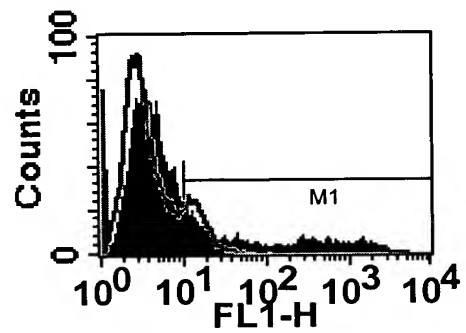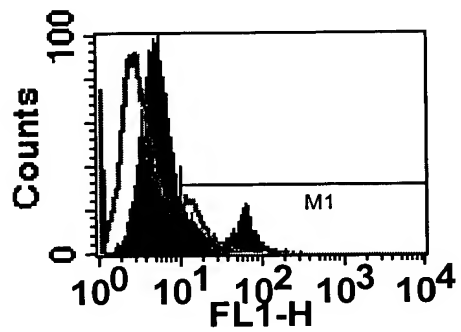
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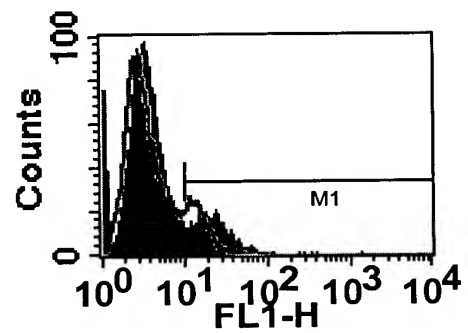
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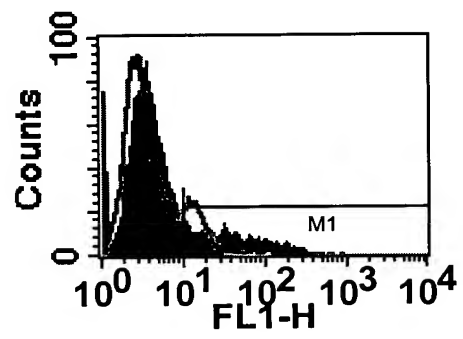Clone 9D8

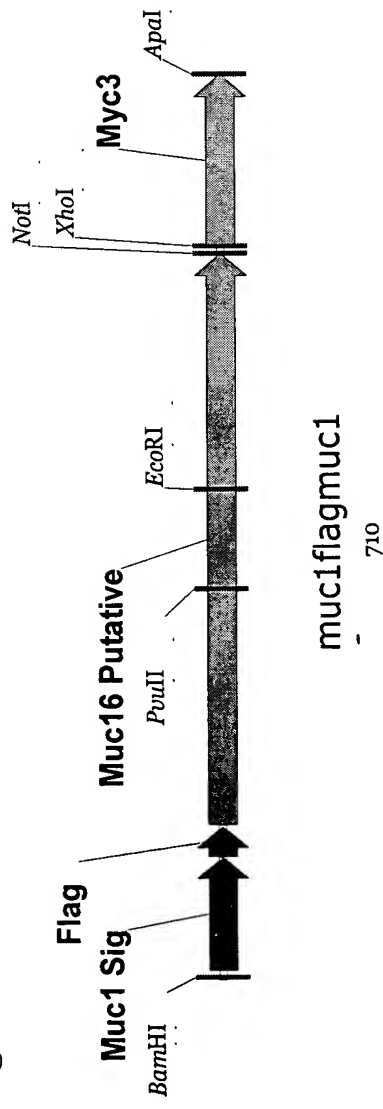Clone 10C9

Clone 2D3

Clone 9G4

Figure 5B

M11

Figure 6A

Flag

Muc1 Sig

BamHI

Muc16 Putative

PvuII

EcoRI

NotI

XhoI

Myc3

ApaI

muc1flagmuc1

710

Figure 6B   (SEQ ID NO:21)

```
     BamHI
     ------
1    GGATCCATGA CACCGGGCAC CCAGTCTCCT TTCTTCCTGC TGCTGCTCCT CACAGTGCTT ACAGTTGTTA CAGGTTCTGG TCATGCAAGC TCTACCGACT ACAAGGACGA
     CCTAGGTACT GTGGCCCGTG GGTCAGAGGA AAGAAGGACG ACGACGAGGA GTGTCACGAA TGTCAACAAT GTCCAAGACC AGTACGTTCG AGATGGCTGA TGTTCCTGCT
              XbaI
              ------

111  CGATGACAAG TCTAGATTCC GAAACAGCAG CATCAAGAGT TATTTTTCTG ACTGTCAAGT TTCAACATTC AGTCTGTCC CCAACAGGCA CCACACCGGG GTGGACTCCC
     GCTACTGTTC AGATCTAAGG CTTTGTCGTC GTAGTTCTCA ATAAAAAGAC TGACAGTTCA AAGTTGTAAG TCAGACAGG GGTTGTCCGT GGTGTGGCCC CACCTGAGGG
                                                                                      KpnI
                                                                                      ------

221  TGTGTAACTT CTCGCCACTG GCTCGGGAGAG TAGACAGAGT TGCCATCTAT GAGGAATTTC TGCGGATGAC CCGGAATGGT ACCCAGCTGC AGAACTTCAC CCTGGACAGG
     ACACATTGAA GAGCGGTGAC CGAGCCTCTC ATCTGTCTCA ACGGTAGATA CTCCTTAAAG ACGCCTACTG GGCCTTACCA TGGGTCGACG TCTTGAAGTG GGACCTGTCC
                                                                  EcoRI
                                                                  ------

331  AGCAGTGTCC TTGTGGATGG GTATTCTCCC AACAGAAATG AGCCCTTAAC TGGGAATTCT GACCTTCCCT TCTGGGCTGT CATCCTCATC GGCTTGGCAG GACTTCCTGG
     TCGTCACAGG AACACCTACC CATAAGAGGG TTGTCTTTAC TCGGGAATTG ACCCTTAAGA CTGGAAGGGA AGACCCGACA GTAGGAGTAG CCGAACCGTC CTGAGGACCC

441  ACTCATCACA TGCCTGATCT GCGGTGTCCT GGTGACCACC CGCCGGCGGA AGAAGGAGGG AGAATACAAC GTCCAGCAAC AGTGCCCAGG CTACTACCAG TCACACCTAG
     TGAGTAGTGT ACGGACTAGA CGCCACAGGA CCACTGGTGG GCGGCCGCCT TCTTCCTCCC TCTTATGTTG CAGGTCGTTG TCACGGGTCC GATGATGGTC AGTGTGGATC
                                  XhoI
                                  ------          NotI
                                                  ---------

551  ACCTGGAGGA TCTGCAAGCG GCCGCTCGAG CCACCATGGA ACAAAAACTC ATCTCAGAAG AGGATCTGGC TAGGCGAACAA AAACTCATCT CAGAAGAGGA TCTGGAACAA
     TGGACCTCCT AGACGTTCGC CGGCGAGCTC GGTGGTACCT TGTTTTTGAG TAGAGTCTTC TCCTAGACCG ATCCGCTTGTT TTTGAGTAGA GTCTTCTCCT AGACCTTGTT
                                                  XbaI              ApaI
                                                  ------            ------

661  AAACTCATCT CAGAAGAGGA TCTGACCGGT TAAATGCATC TAGAGGGCCC
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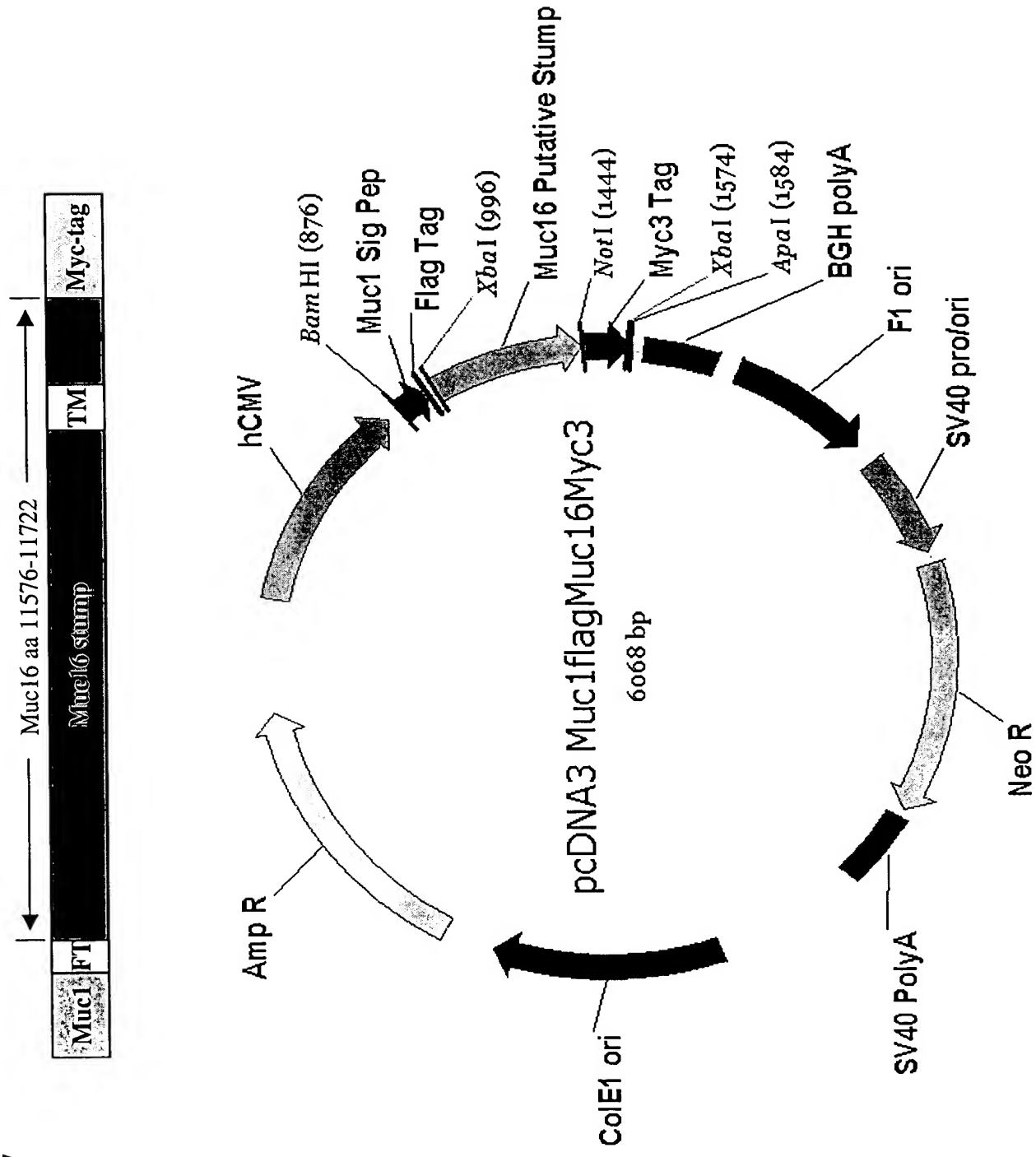
Figure 7

Figure 8

Recombinant Muc16 Stump Protein

α-Flag

α-Myc

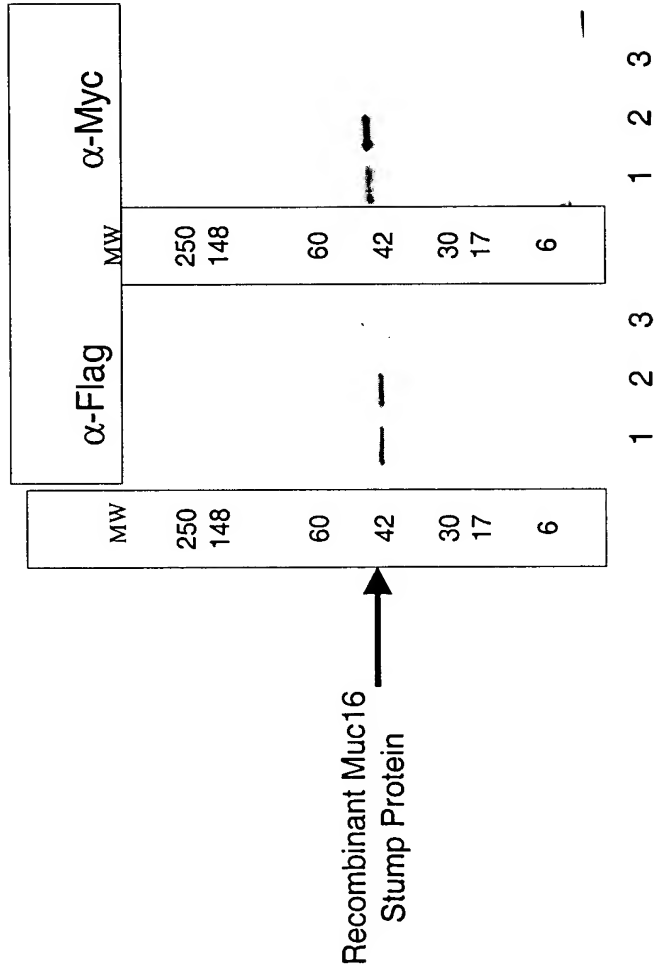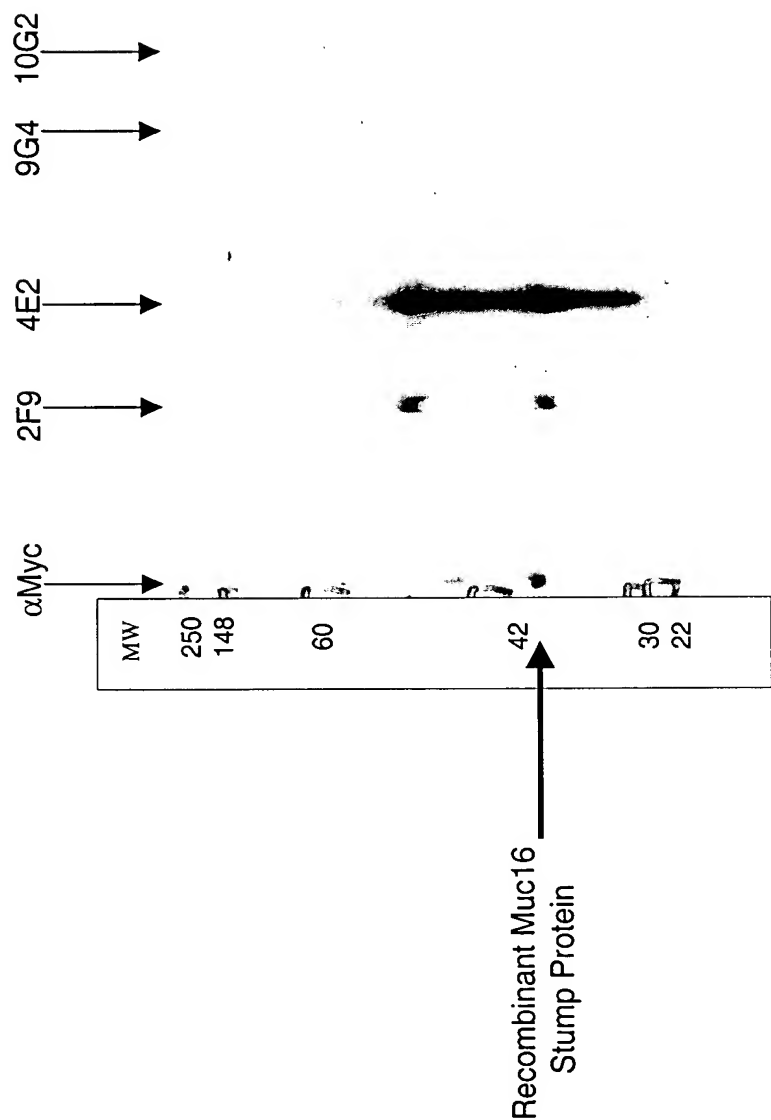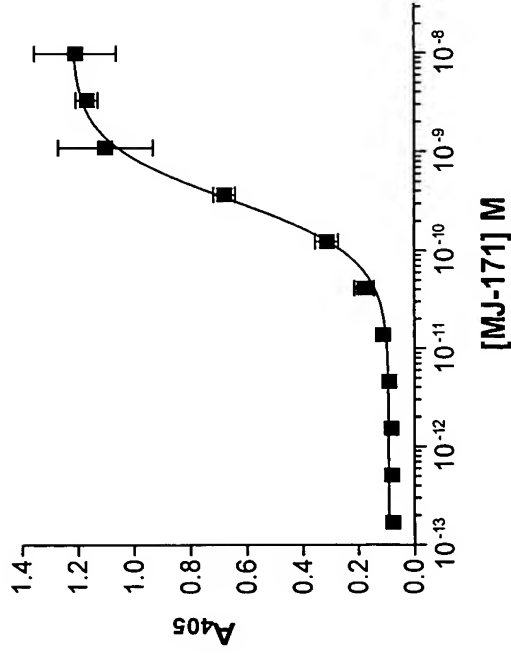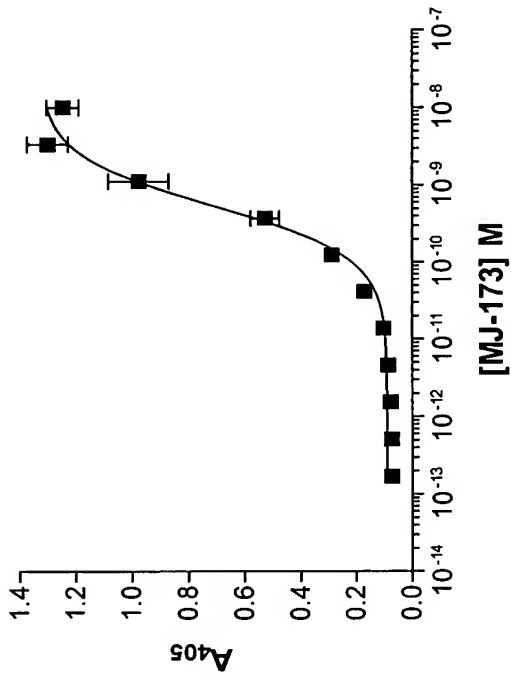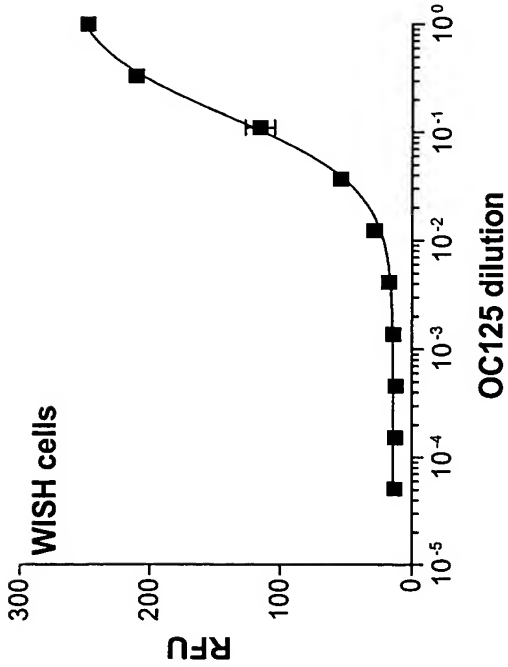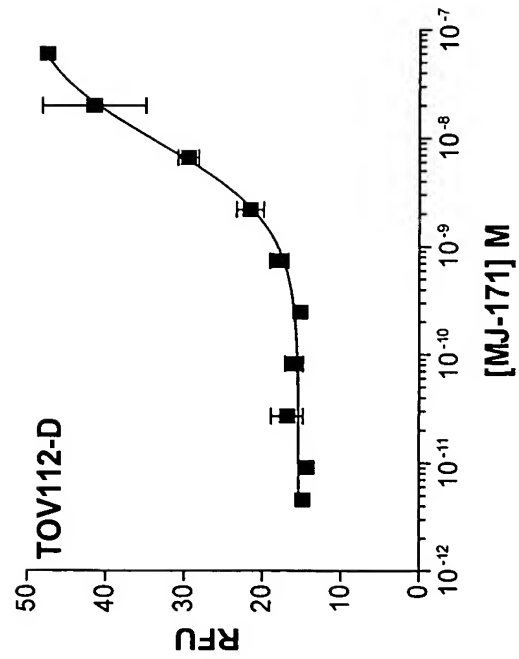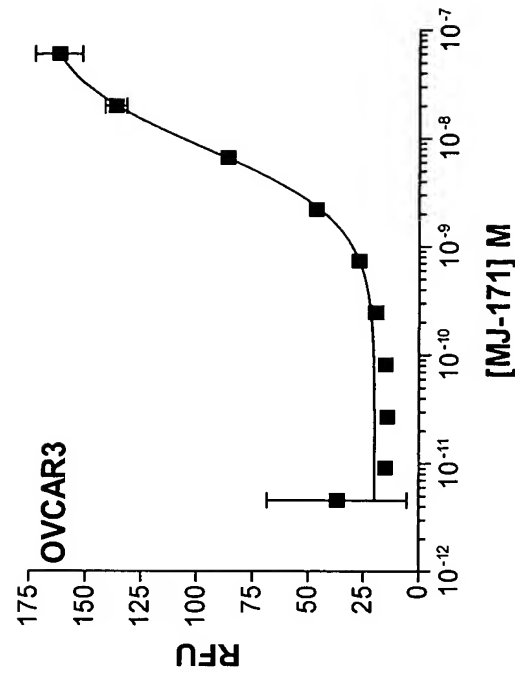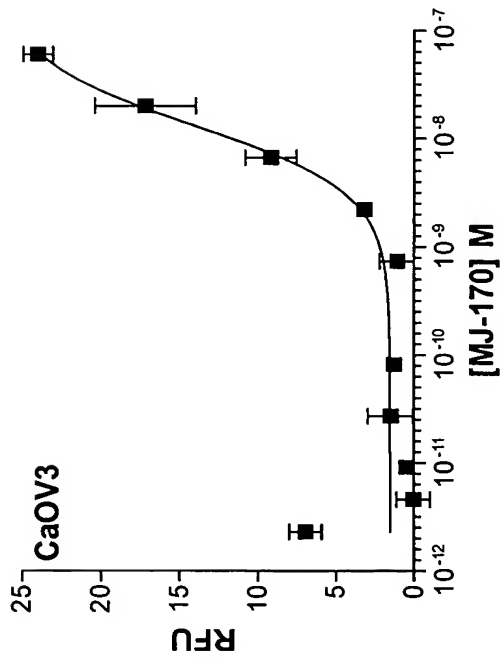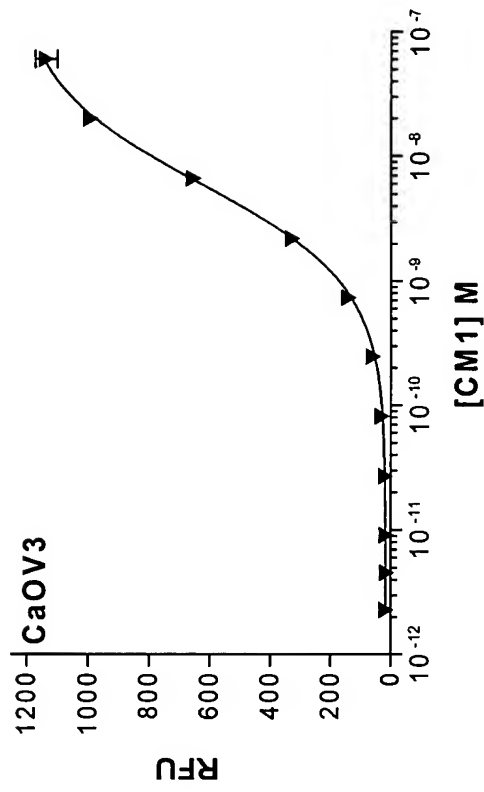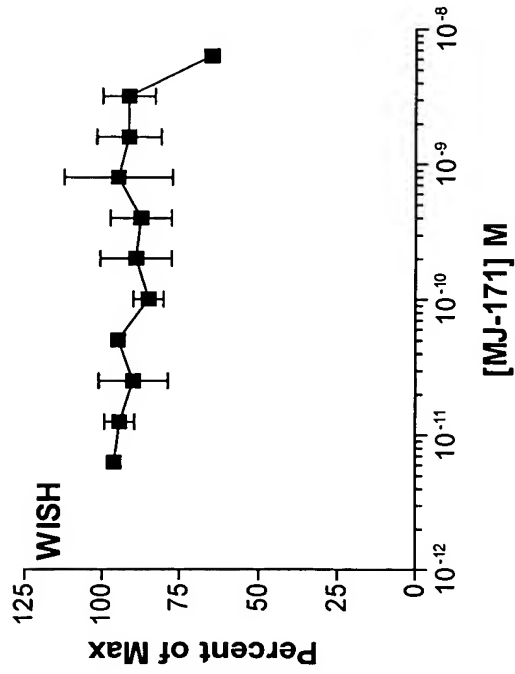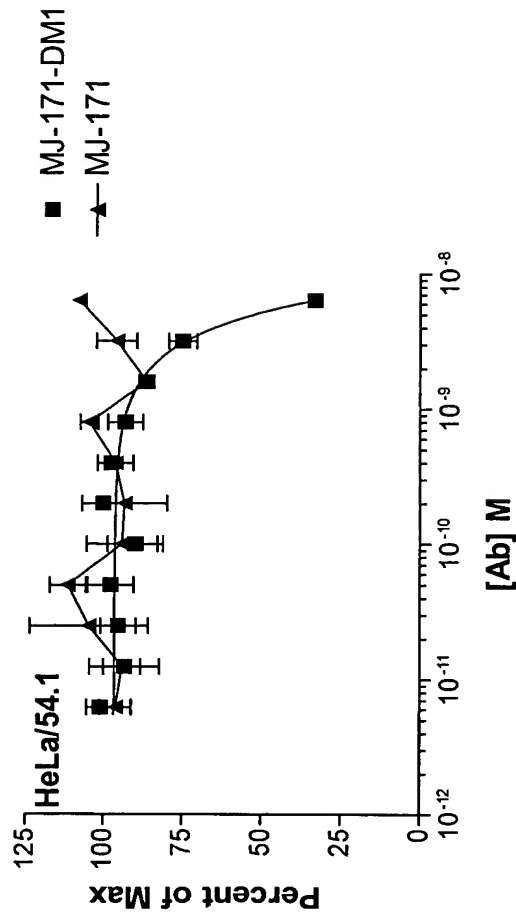| MW			MW		
250			250		
148			148		
60			60		
42			42		
30			30		
17			17		
6			6		

1 2 3   1 2 3

Figure 9

Figure 10A

Figure 10B

Figure 10C
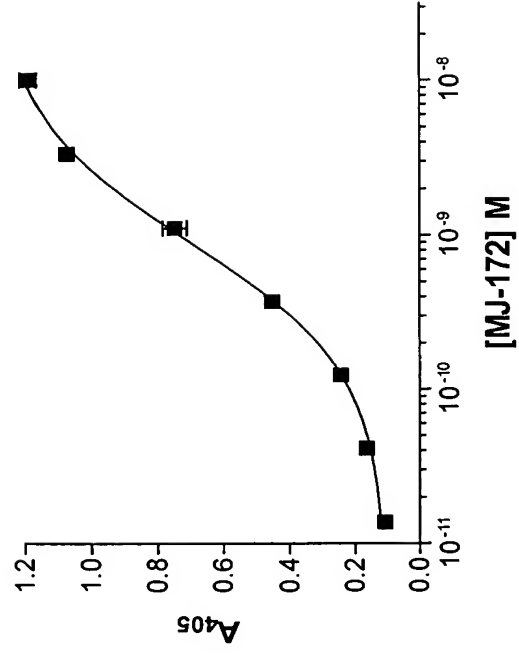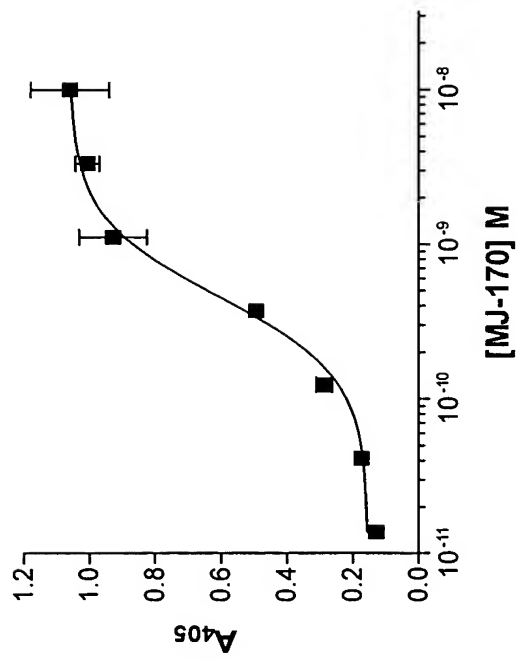
Figure 11

Figure 12A

WISH cells

OC125 dilution

Figure 12B

WISH

[MJ-171] M

Figure 12C

SkBr3

[MJ-171] M

Figure 12D

OV90

[Ab] M

■ MJ-173
▲ MJ-171

Figure 12F

OVCAR3

Figure 12E

PA1

Figure 12G

TOV112-D
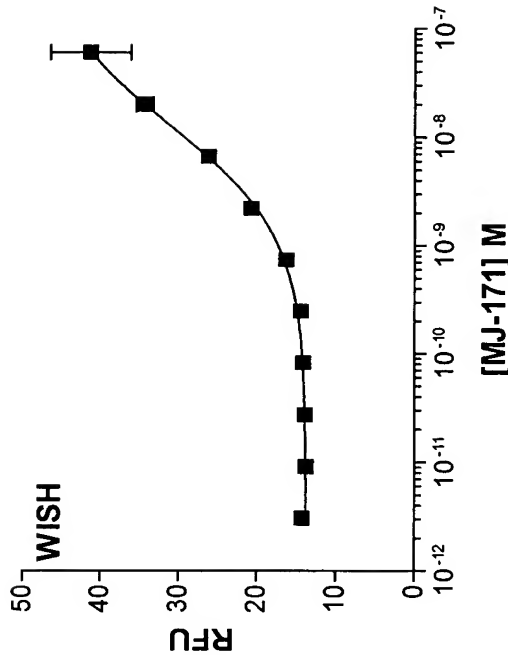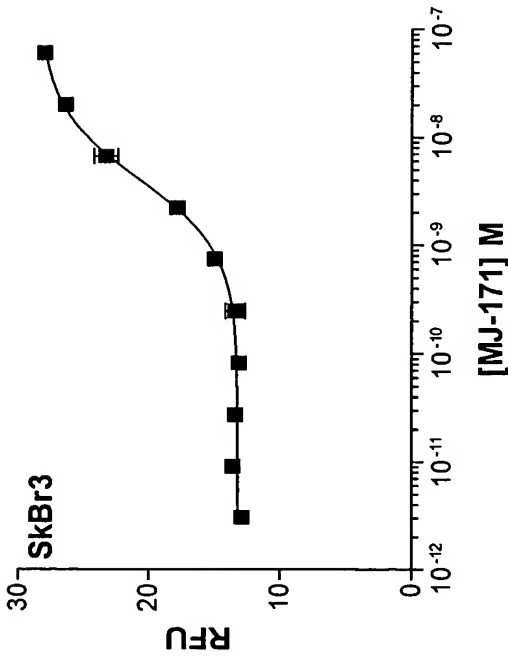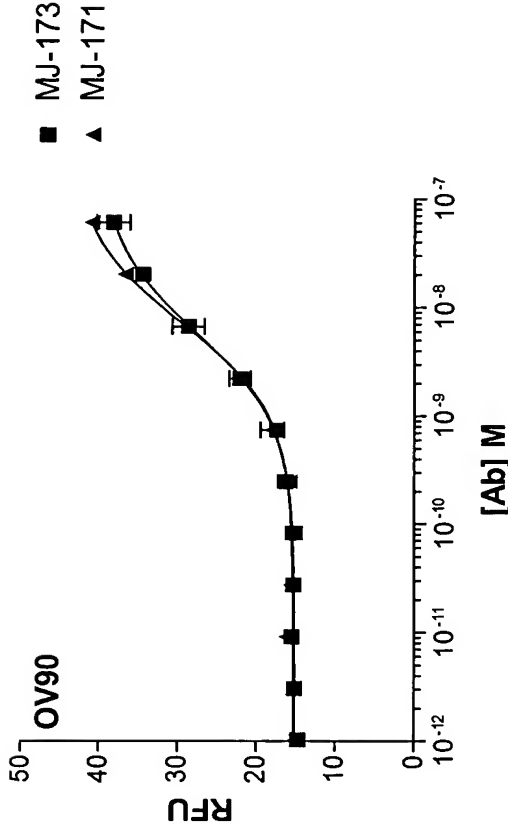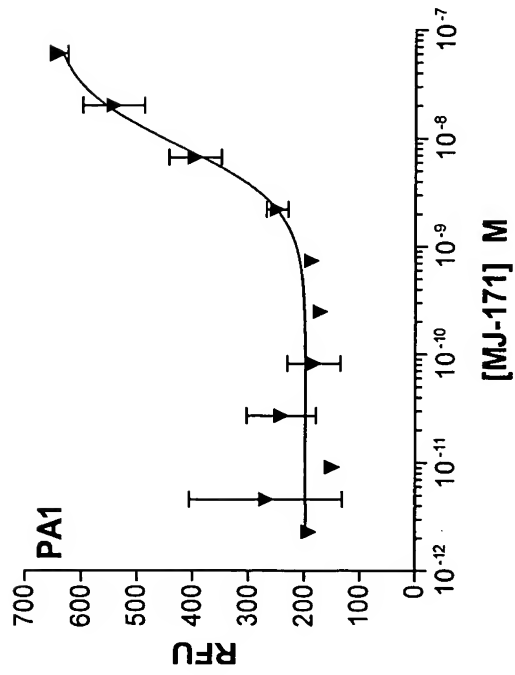
Figure 13A

Figure 13B

Figure 14A

WISH

Figure 14B

PA1

Figure 14C

HeLa/54.1

- ■— MJ-171-DM1
- —▲— MJ-171

Figure 15

CaOV3

$IC_{50} = 1.6e-9$ M

[MJ-170-DM1] M

% of Max

Figure 16

Figure 17

Surviving fraction vs [MJ-170-DM1] M

CaOV3

~IC_{50} 3.4e-10 M